REVIEW

5-Azacytidine

A New Anticancer Drug with Effectiveness in Acute Myelogenous Leukemia

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Clinical studies involving 5-azacytidine, a ring analogue of cytidine, began in Europe in 1967 and the United States in 1970, and we review available preclinical and clinical studies here. The drug possesses cytotoxic, antimicrobial, antineoplastic, abortive, and mutagenic activity in various biological systems. 5-Azacytidine is thought to exert its antineoplastic effect through interference with nucleic acid metabolism. The dose-limiting toxicities are nausea, vomiting, and leukopenia, while the incidence of thrombocytopenia is low. Hepatic toxicity ranges from abnormal findings in liver function tests to hepatic coma. Clinical results in solid tumors are not encouraging, but 5-azacytidine shows consistent antitumor activity in patients with acute myelogenous leukemia resistant to previous treatment. An overall response rate of 35%, with 20% complete remissions, was achieved in 200 previously treated patients with acute myelogenous leukemia. Further studies must define the role of 5-azacytidine alone and in combination for the first-line treatment of acute myelogenous leukemia.

AMONG THE INVESTIGATIONAL ANTICANCER DRUGS currently being sponsored for clinical trial by the Division of Cancer Treatment, National Cancer Institute, is 5-azacytidine, a ring analogue of cytidine. It was synthesized by the Czechoslovakian investigators Piskala and Sorm in 1964 (1) and produced microbiologically by Hanka and colleagues (2) in 1966.

Clinical trials in Europe using 5-azacytidine as an anticancer agent were begun in 1967. Phase I clinical trials in the United States were begun in late 1970. During the last 5 years, cooperative chemotherapy groups and independent investigators have been studying 5-azacytidine in patients with advanced metastatic cancer and leukemia refractory to conventional chemotherapeutic agents. Although the drug has been used in the clinic for more than 5 years, no comprehensive review has been undertaken.

We summarize here the preclinical and clinical data from published as well as unpublished reports. This information should help bring into focus the potential clinical usefulness of 5-azacytidine in the treatment of acute myelogenous leukemia.

Drug Information

BIOCHEMISTRY

5-Azacytidine (4-amino-1-β-D-ribofuranosyl-1,3,5 triazine-2-one or 1-β-D-ribofuranosyl-5-azacytosine) was synthesized in 1964 (1). Two years later it was produced microbiologically in a fermentation beer of *Streptoverticillium ladakanus* var. *ladakanus* (2), with further characterization of the compound by Bergy and Herr (3). Structurally, 5-azacytidine is a ring analogue of the pyrimidine nucleoside cytidine and differs from it only by a nitrogen in place of the fifth carbon (*see* Figure 1, with cytidine shown for comparison). The identifying chemical properties of the compound are detailed elsewhere (3).

GENERAL BIOLOGICAL EFFECTS

5-Azacytidine was exciting to early investigators because it possessed antimicrobial (2), cytotoxic (2), antineoplastic (2), abortive (4), mutagenic (5-8), and leukopenic (9) activity. Other intriguing properties, as yet incompletely investigated, include a protective effect on bacteria exposed to ultraviolet light (10) and on mice exposed to lethal X-irradiation (11). 5-Azacytidine is also a strong inhibitor of antibody formation (12) and is considered immunosuppressive in mice (13).

MECHANISM OF ACTION

As an antimetabolite, 5-azacytidine is thought to exert its antineoplastic effect through an interference with nucleic acid metabolism. The precise steps in nucleic acid metabolism affected have not yet been identified. Four mechanisms for this interference have been proposed: (1) incorporation of 5-azacytidine into DNA (14); (2) incorporation of 5-azacytidine into RNA (15) and hence interference with

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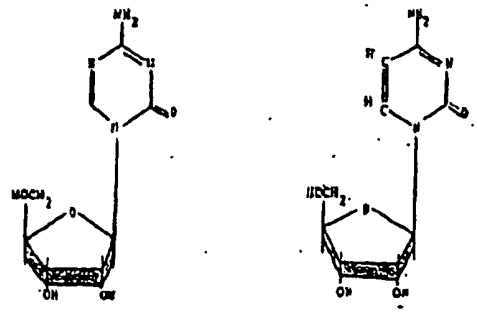

5-azacytidine cytidine

Figure 1. Structure of 5-azacytidine, with cytidine shown for comparison.

protein synthesis; [3] competition for uridine kinase (16); and [4] inhibition of orotidylic acid decarboxylase (17, 18). There is supportive evidence for each of these proposed actions.

5-Azacytidine is phosphorylated and incorporated into both DNA and RNA polynucleotides of various bacterial and animal tumor systems (15, 19-26). Spontaneous degradation of the symmetrical triazine molecule of 5-azacytidine makes the DNA less stable and more liable to disruption of the secondary structure and hence causes chromosomal breakage (14, 21, 27-29).

The incorporation of 5-azacytidine into newly synthesized messenger RNA (29, 30) gives defective messenger RNA that cannot code properly for protein synthesis, and hence there is inhibition of protein synthesis. Transfer RNA synthesized in the presence of 5-azacytidine is also structurally and functionally modified, and such modification may be one of the major actions of 5-azacytidine in inhibiting protein synthesis in mammalian cells (31).

Uridine kinase, the enzyme that catalyzes the phosphorylation of uridine and cytidine (32), is regarded as the rate-limiting step in the pyrimidine salvage pathway (33, 34). It is the first enzyme taking part in the metabolic change of 5-azacytidine (35). 5-Azacytidine probably competes with uridine and cytidine for uridine kinase, since uridine and cytidine will reverse the bacteriostatic effects of 5-azacytidine (16).

5-Azacytidine also blocks the *de novo* synthesis of pyrimidines by interfering with orotidylic acid decarboxylase (17).

ACTIVITY IN EXPERIMENTAL TUMORS

Sorm and Vesely (9) reported the activity of 5-azacytidine against lymphoid leukemia in AK mice in 1964. Since then, the drug has shown significant activity in the L1210 mouse leukemia system and was active in sublines of L1210 resistant to 6-mercaptopurine, aminopterin, and cyclophosphamide (2).

5-Azacytidine has been shown ineffective in animal solid tumor systems, including Walker 256 and spontaneous mammary tumor (36). The globulin secreted from murine myeloma tumor MOPC 21 in the presence of 5-azacytidine was still intact but decreased in amount (37). Combinations of 5-azacytidine and emetine have shown enhanced activity in the L1210 system (38). 5-Azacytidine and cytosine arabinoside have also been synergistic in that system when used in the appropriate schedule (39).

The question of drug schedule dependency in the various tumor systems is a controversial matter. Venditti (40) claims that 5-azacytidine has no important schedule dependency because daily and intermittent treatment schedules in L1210 leukemia-bearing mice are equally effective. However, when individual hamster fibroblasts are examined, the effect of 5-azacytidine seems to be cell-cycle phase specific in that it is most toxic to cells in the S phase, especially at low concentrations (41, 42). Lloyd, Culmadge, and Wilkoff (43) also conclude that 5-azacytidine is a cell-cycle phase specific agent, since nonproliferating L1210 cells are relatively insensitive to concentrations of 5-azacytidine that markedly reduce the viability of proliferating cultured L1210 cells.

ANIMAL TOXICOLOGY

Four animal species (hamsters, mice, Rhesus monkeys, and beagle dogs) have been studied for single and repeated dose toxicity. The most sensitive species appears to be the beagle (44, 45). 5-Azacytidine caused a moderate decrease in the level of circulating leukocytes. The platelets seemed to be the least susceptible of the blood elements. Widespread necrosis of lymphatic organs was seen. There were no detrimental effects on gastrointestinal epithelium (45).

Single doses of 5-azacytidine of 8 mg/m² body surface area were damaging to the liver of the dogs, with elevations of alkaline phosphatase, serum glutamic pyruvate transaminase, and prothrombin time noted. The principal morphologic effect on renal tissues was the accumulation of a globular eosinophilic material in the lumens of the renal tubules. Blood urea nitrogen levels were occasionally elevated (45). Overall, the toxicity of 5-azacytidine in the beagle appeared predictable, dose-related, and reversible in most instances. An exception was hepatic cell necrosis, which occurred sporadically.

METABOLISM AND PHARMACOKINETICS

In-vitro studies of the drug show that it undergoes rapid hydrolysis in neutral and basic media (46). The degradation products do not produce as much bacteriostatic activity as does 5-azacytidine (46) but are currently being investigated for antitumor activity.

Rasko and associates (18) studied the pharmacokinetics of the drug in mice using ^{14}C-labeled 5-azacytidine. The radioactivity, which could represent parent drug or metabolite(s), dropped rapidly in the blood during the first 8 h after administration, and the calculated plasma half-life was approximately 3.8 h. The level of radioactivity was greater and the retention longer in the lymphatic organs. The relatively rapid drop in concentrations of radioactivity in the serum corresponded to initial rapid excretion in the urine.

Elegant pharmacokinetic studies in man by Troetel and associates (47) described the distribution and excretion of radioactively labeled 5-azacytidine given by intravenous and subcutaneous routes. The absorption from the subcutaneous site was rapid, with plasma levels within 2 h

Table 3. Phase I Studies with 5-Azacytidine

Dose Schedule	Dosage			Cancer Studied		Reference*
	Starting	Higher Escalation	Maximally Tolerated Dosage	Solid Tumor	Acute Leukemia	
	mg/m² body surface					
Weekly intravenously	200	600	300	Yes		EST (53)
Twice a week intravenously	50	200	150	Yes		SEG (48)
Daily intravenously × 5 every 14 days	50	300	150-200		Yes†	CCA (52)
Daily intravenously × 5, 9 days off	50	268	150	Yes		EST (53)
Daily intravenously × 5 every 21 days	2.2	300	175-225	Yes	Yes	McCredie (54)
Daily intravenously × 5 every 14 days	400	400	300		Yes	McCredie (55)
Daily intravenously × 10 days	1.11‡	89‡	59‡	Yes		COG (56)
Infusion over 5 days every 14 days§	50	400	150-200	Yes	Yes	SEG (57)
Infusion over 120 hours every 28 days‖	150	200	150	Yes		Lomen (58)

* EST = Eastern Cooperative Oncology Group; SEG = Southeastern Cooperative Oncology Group; CCA = Children's Cancer Study Group; COG = Central Oncology Group.
† Child patients.
‡ Calculated by conversion factor of 37.
§ Fresh solution every 3 to 12 h.
‖ Fresh solution every 4 h.

equal to that noted in patients treated with intravenous drug. The plasma half-life for radioactively labeled 5-azacytidine and its radioactive metabolites after intravenous injection was 3.5 h and after subcutaneous injection was 4.2 h. This was confirmed in subsequent studies by Vogler, Arkun, and Velez-Garcia (48), who found a half-life of 3 h to 4.7 h.

Patients receiving the drug excreted 90% of the total radioactivity in the urine within 24 h (49). Drug was taken up into tumor tissue, and radioactivity in the tumor was always greater than that in the normal surrounding tissue (47). However, no active transport mechanism has been shown. Radioactivity was demonstrated in ascitic fluid but did not reach equilibrium until 6 h after administration (47). The concentration of radioactivity in the cerebrospinal fluid was measured 24 h after administration and was equivalent to 0.2 μg of drug/ml of cerebrospinal fluid. No significant radioactivity was found in feces, sputum, vomitus, or expired carbon dioxide (47, 49).

DRUG SUPPLY AND PHARMACEUTICAL DATA

5-Azacytidine is presently available only as an investigational drug through the Investigational Drug Branch of the Cancer Therapy Evaluation Program. The product is supplied as a white lyophilized powder in vials containing 100 mg of 5-azacytidine with mannitol, USP, 100 mg. When reconstituted with 19.9 ml of sterile water for injection, USP, each milliliter of solution contains 5 mg of 5-azacytidine and 5 mg of mannitol. The pH of the solution will be 6.0 to 7.5. The reconstituted solution hydrolyzes at room temperature and should be used within 30 min.

The reconstituted solution can be further diluted in lactated Ringer's injection, USP, which provides the optimum pH (6.37) for solution stability. Reconstituted solutions that are further diluted with lactated Ringer's injection should be used within 2 to 3 h. During this time there is a 10% to 15% decomposition of the 5-azacytidine based on ultraviolet and nuclear magnetic resonance assay methods. Drug potency is jeopardized if the solution is not used within that period of time (50).

Results of Clinical Trials

This review of clinical trials encompasses a total of 58 protocols received and reviewed at the Investigational Drug Branch. To date this represents 521 evaluable patients who have received the drug.

TERMINOLOGY

The patients reported include only those who received 5-azacytidine by a predetermined dose schedule and who could be followed by an objective index of disease (evaluable patients).

Partial remission denotes a greater than 50% measurable decrease in tumor area, lasting a minimum of 1 month, or the occurrence of an M_2* marrow in the case of leukemias. Complete remission denotes complete disappearance of measurable disease or an M_1* marrow.

EUROPEAN TRIALS

In 1971 Hrodek and Vesely (51) first reported that intramuscular 5-azacytidine had activity in childhood leukemia. When 5-azacytidine was used alone during the induction phase, there was a decrease in the leukocyte count, but complete remissions were obtained only with the addition of prednisone. These results were very encouraging, with about an 88% response rate in patients with acute lymphocytic leukemia. Unfortunately, one could not sort out what role 5-azacytidine had in these remissions.

STUDIES IN THE UNITED STATES

The first Phase I studies in the United States were begun in late 1970. Based on the toxicity results in dogs, the initial starting dose in man was estimated to be 3.3 mg/m²·day for 5 days. Karon and colleagues (52) started out at

* Criteria for remission of leukemia of the cooperative study groups. M_1 denotes fewer than 5% blast cells or other leukemic cells in a normally cellular bone marrow, and M_2 denotes 5% to 25% leukemic cells in the bone marrow.

Table 2. 5-Azacytidine Therapy Alone in Acute Myelogenous Leukemia

Reference*	Dose	Dose Schedule	Evaluable Patients	Complete Remissions	Partial Remissions
	mg/m² body surface		no.	no. (%)	no. (%)
SWOG (59)	750	Single intravenously	27	11 (41)	4 (15)
CCA (52)	150-200†	Daily intravenously × 5	14	5 (36)	1 (7)
McCredie (55)	150-400	Daily intravenously × 5	18	3 (17)	4 (22)
Tan (60)	120-300	Daily intravenously × 5	6	0 (0)	1 (17)
Levi (61)	200	Daily intravenously × 5 in 3 divided doses	18	5 (28)	1 (5)
WCG (62)	60	Every 8 h × 15 doses	6	0 (0)	0 (0)
	100	Every 8 h × 15 doses	26	2 (8)	7 (27)
	180	Daily intravenously × 5	8	2 (25)	0 (0)
	300	Daily intravenously × 5	26	2 (8)	7 (27)
SEG (57)	150-200	Infusion daily × 5‡	22	7 (28)	3 (12)
McCredie (59)	500	Daily intravenously × 5§	29	4 (14)	4 (14)

* SWOG = Southwest Oncology Group; CCA = Children's Cancer Study Group; WCG = Western Cancer Study Group; SEG = Southeastern Group-ative Oncology Group.
† Average dosage in Phase I trial.
‡ Change solution every 3 h.
§ Infusion over 30 min.

2 mg/m² · day, but, having found no toxicity after several dosage escalations (Fibonacci scale), they increased the dose 70 to 100 times the initial starting dose before encountering toxicity. Other investigators explored either the same or different dosage schedules.

The Phase I studies that have involved 207 patients to date are summarized in Table 1 (53-58), which includes the starting dose, highest escalated dose, and maximally tolerated dose for each study. The dose-limiting toxicities have consistently been nausea, vomiting, and leukopenia (*see* Toxicity).

The maximally tolerated dosages on a daily times five schedule have been 150 to 200 mg/m² · day for 5 days every 14 to 21 days, depending on recovery from myelosuppression. The maximally tolerated dose for a weekly intravenous dosage has been about 500 mg/m² and 150 mg/m² for a twice weekly schedule. When continuous intravenous infusions have been used, the maximally tolerated dose has been 150 to 200 mg/m² · day for 5 days every 14 to 28 days, with infusion solutions being changed every 3 to 4 hours to avoid loss of drug potency secondary to drug decomposition (50). The continous infusion may have an advantage in producing less gastrointestinal intolerance (vide infra).

ANTINEOPLASTIC ACTIVITY BY TUMOR TYPE

Acute Myelogenous Leukemia: Despite recent advances, the treatment of acute myelogenous leukemia remains much less effective than that of acute lymphocytic leukemia. Most current treatment regimens for acute myelogenous leukemia include combinations of cytosine arabinoside, 6-thioguanine, daunomycin, and occasionally adriamycin, and vincristine or prednisone, or both. Patients who have failed on these agents have had little hope for remissions. 5-Azacytidine appears to be a useful agent for some of the cases refractory to these drugs.

The effectiveness of 5-azacytidine has emerged primarily in studies in acute myelogenous leukemia. The results on a total of 200 acute myelogenous leukemia patients treated with 5-azacytidine alone showed an overall response rate of 36% (20% complete remissions and 16% partial remissions (*see* Table 2 (59-62)). The median duration of remissions has been between 15 and 19 weeks. The most striking point in these statistics is that almost all of the patients who responded had been refractory to all conventional antileukemic agents.

It is difficult to draw any conclusions as to which is the most effective dose schedule, since approximately equal remission rates have been seen with 100 to 250 mg/m² twice weekly, 150 to 400 mg/m² · day times 5 days, and continous infusions of 5-azacytidine over 5 days. However, certain schedules may be advantageous in terms of toxicity (see Toxicity).

Sixty-six more patients with acute myelogenous leukemia have been treated with 5-azacytidine in combination with one or more other chemotherapeutic agents in Phase II trials (*see* Table 3 (63-65)). Response rates with combinations including 5-azacytidine have ranged from 29% to 68%, but the number of patients treated in each study has been too small for any meaningful conclusions.

In summary, 5-azacytidine has been effective in inducing remissions in some refractory cases of acute myelogenous leukemia, and further combinations of 5-azacytidine with other drugs are being explored in previously treated patients. The next step in the evaluation as an antileukemic agent will be to use 5-azacytidine in combinations as first-line treatment of acute myelogenous leukemia.

Acute Lymphocytic Leukemia: Table 4 (66-70) shows the American experience with acute lymphocytic leukemia, which has not been as encouraging as the earlier European experience. Of 49 refractory patients treated with 5-azacytidine alone, there have been only three complete remissions and two partial remissions, for a 10% overall response rate.

Chronic Myelogenous Leukemia: There have been no remissions in nine patients treated with 5-azacytidine, but three did show hematologic improvement.

Multiple Myeloma: No responses were seen in nine patients evaluated on a 5-azacytidine and prednisone combination (66).

While 5-azacytidine has given encouraging results in the treatment of acute myelogenous leukemia, the drug has not yet shown any significant activity in solid tumors. Table 4 summarizes the single agent activity of 5-azacytidine in various types of cancer. Some of the solid tumors that have been evaluated include:

Breast: Weiss and colleagues (56) reported that seven of 11 patients with breast cancer responded to 5-azacytidine in early Phase I trials. Most of these patients had either chest wall recurrences or unresectable tumors involving the breast. One remission did last 6 months. This encouraging antitumor activity was not confirmed by others, with the exception of Bellet and associates (69), who did notice an early, dramatic partial remission in one of four evaluable breast cancer patients.

Of 75 patients with metastatic breast cancer given 5-azacytidine, the response rate was 19%. From these results it must be concluded that 5-azacytidine is not an active agent in breast cancer as was originally expected. However, it should be noted that most of the patients studied had been refractory to other chemotherapy.

Lung: Forty-five patients have been treated in Phase I and II studies, with a total of seven partial responses. No particular cell type has predominated. Because of the low activity and accompanying gastrointestinal intolerance, further trials in this disease are not contemplated.

Colon and Rectum: Eighty-one patients have been treated, with a total of five partial remissions. The drug does not seem to offer any therapeutic benefit in this disease (70).

Melanoma: Forty-four patients with malignant melanoma have been treated with 5-azacytidine. Three patients have had a partial response, with one remission of 6 months' duration.

Miscellaneous Tumors: Several other tumor types detailed in Table 4 have had inadequate trials with 5-azacytidine; thus, no statement can be made regarding the presence or lack of antitumor effect. Of interest, however, have been a few partial responses in ovarian cancer, lymphoma, and mesothelioma.

Toxicity

Table 5 summarizes all of the major toxicities in the Phase I, II, and III clinical trials reported to the Investigational Drug Branch. This table includes information on 745 patients who received 5-azacytidine alone at various dosage levels. The major toxicities have been gastrointestinal, hematologic, and hepatic.

GASTROINTESTINAL

Nausea and vomiting have been particularly troublesome toxicities, with overall incidences of approximately 73%. The nausea and vomiting begin 1½ to 3 h after intravenous injection and usually recur with each subsequent injection (48, 69). Antiemetics such as chlorpromazine have had variable effect on these symptoms, with some investigators believing them to be useful if the patient is premedicated for 24 to 48 h before the course of 5-azacytidine is given (52). Because of the nausea and vomiting, some patients have been unwilling to have repeated courses of the drug (68). This certainly is a major factor against further trials with the drug in conditions where its activity has been borderline.

A number of investigators noted a slight decrease in the severity of the nausea and vomiting if the drug was given subcutaneously, in divided doses intravenously, or by a fast drip over 15 min (52, 55, 56, 60, 69). Two protocols were designed by Vogler, Miller, and Keller (57) and Lonsen, Vaitkevicius, and Samson (58) to give the drug by continuous infusion over a 5-day period. An objection to this method is that the activity of 5-azacytidine (as measured in physicochemical and biological systems) is less than anticipated because of degradation while in the infusion solution (*see* Drug Supply and Pharmaceutical Data). One can circumvent this problem by having infusion solutions made in Ringer's lactate, USP, and changing the bottles with new solutions hung every 3 to 4 h (57, 58). Nausea and vomiting reported with the infusion data is reduced to 50% to 70% in Vogler's series (57) and to a virtual absence of it in Lonsen's series (58).

The average reported incidence of diarrhea in all series is 53%. It has not been a dose-limiting toxicity. The infusion, or subcutaneous route, may also decrease its incidence.

HEMATOLOGIC

Several of the Phase I studies pointed to leukopenia as a dose-limiting toxicity. The overall incidence of leukopenia (less than 1500/mm³ total leukocyte count) was 34%, including all dosage levels. It was a dose-related phenomenon.

In patients with solid tumors treated on a daily intravenous times five schedule, the mean nadir of leukopenia has

Table 3. Combination Therapy of Acute Myelogenous Leukemia with 5-Azacytidine plus Other Agents

Reference*	Drug Combination	Evaluable Patients	Complete Remissions	Partial Remissions
		no.	no. (%)	no. (%)
SWOG (59)	5-Azacytidine + adenosine arabinoside	14	4 (29)	0 (0)
CCA (63)	5-Azacytidine + daunomycin	19	11 (58)	2 (10)
Levi (64)	5-Azacytidine + methylglyoxal-bis-guanylhydrazone	8	0 (0)	1 (13)
Tan (65)	5-Azacytidine + adriamycin + bleomycin†	1	0 (0)	1 (100)
CCA (63)	5-Azacytidine + cytosine arabinoside + prednisone + vincristine‡	24	12 (50)	4 (17)

* SWOG = Southwest Oncology Group; CCA = Children's Cancer Study Group.
† Given as result of counts.
‡ This regimen has recently been changed to include daunomycin.

Table 4. Summary of Single-Agent Activity of 5-Azacytidine in Various Tumor Types

Tumor Type	Evaluable Patients	Complete Remissions	Partial Remissions	References
	no.	no. (%)	no. (%)	
Acute myelogenous leukemia	200	41 (20)	32 (16)	40, 52, 55, 57, 59-62
Acute lymphocytic leukemia	49	3 (6)	2 (4)	52, 55, 60
Chronic myelogenous leukemia*	9	0 (0)	0 (0)	55, 57, 60
Multiple myeloma	9	0 (0)	0 (0)	66
Breast cancer	75	0 (0)	14 (19)	48, 53, 56, 67-69
Lung cancer†	45	0 (0)	7 (16)	48, 53, 56, 67, 69
Colorectal cancer	81	0 (0)	5 (6)	48, 56, 58, 69, 70
Malignant melanoma	44	0 (0)	3 (7)	48, 53, 56, 60, 67, 69
Miscellaneous tumors				
Ovarian cancer	11	0 (0)	2 (18)	56, 67, 69
Lymphoma‡	7	0 (0)	2 (29)	53, 56, 58, 60
Mesothelioma	2	0 (0)	1 (50)	48
Renal cell carcinoma	17	0 (0)	2 (12)	53, 67, 69
Testicular carcinoma§	1	0 (0)	1 (100)	67
Pancreatic carcinoma	7	0 (0)	1 (14)	48, 53, 56, 67
Esophageal carcinoma	2	0 (0)	0 (0)	48, 67
Stomach carcinoma	10	0 (0)	1 (10)	48
Cervical carcinoma	3	0 (0)	0 (0)	53
Endometrial carcinoma	3	0 (0)	0 (0)	48, 53
Glioblastoma	2	0 (0)	0 (0)	53
Head and neck cancer‖	6	0 (0)	0 (0)	53, 67, 69
Soft tissue sarcoma†	10	0 (0)	0 (0)	48, 53, 67
Hepatoma	3	0 (0)	0 (0)	48, 53, 67
Bladder cancer	1	0 (0)	0 (0)	67
Skin cancer	1	0 (0)	0 (0)	48
Chordoma	1	0 (0)	0 (0)	69

* These patients did have hematologic improvement.
† All cell types.
‡ All histologic types.
§ Embryonal cell.
‖ Cancer of tongue (1 case); parotid tumor (1 case); cancer of larynx (1 case); cancer of tonsil (1 case); and cancer of epiglottis (1 case).

been on Day 25, with a range of 18 to 30 days (70). The nadir has been slightly later on a daily intravenous times 10 schedule (70). Recovery usually takes place in 1 to 3 weeks (48, 61, 69), but, as usual, vigorous support in the leukopenic period has been necessary (70). Subcutaneous administration over 10 days also seems to delay the leukopenia somewhat (21 to 35 days past the start of treatment). Hematotoxicity does not appear to be cumulative in patients with solid tumors.

In patients with acute nonlymphocytic leukemia treated on a daily intravenous 5-day schedule, the median time to nadir of leukopenia has been 14 to 17 days, with a range of 2 to 30 days (61). The median duration of the nadir was 14 to 17 days (range, 2 to 79) (52, 61), and occasionally the leukopenia was prolonged (55).

Thrombocytopenia (less than 100 000/mm³) has been reported in 17% of the patients receiving the drug. Its mean nadir occurs in 14 to 18 days, with a range of 13 to 21 days (61, 70). The median duration of the nadir has been 15 days. The thrombocytopenia is dose-dependent and of surprisingly low incidence; however it may be fatal (69), and platelet support should be available when 5-azacytidine is given.

Anemia has not been a great problem, with approximately 4% of patients having a greater than 3-g/dl drop in hemoglobin directly attributable to the drug.

HEPATIC

The overall incidence of abnormal findings in liver function tests thought to be secondary to 5-azacytidine is about 7%. Hepatic coma became manifest in 0.5% of patients receiving the drug. The hepatotoxicity does not seem to be related to dose, schedule, or route of administration, and therefore one must be wary of using the drug in the presence of liver disease. Bellet and co-workers (71) have reported four deaths secondary to hepatic coma in patients receiving 5-azacytidine who had preexisting liver derangement. When they compared the patients dying in hepatic coma and a surviving group with hepatic metastases, the former had a baseline serum albumin of less than 2.8 g/dl, while the latter had albumins greater than 3.0 g/dl. Percutaneous liver biopsies in patients with metastases could not be evaluated before or after treatment because the biopsies consisted mostly of tumor. Bellet and co-workers concluded that 5-azacytidine is contraindicated in patients with hepatic metastases and serum albumin levels less than 3 g/dl.

NEUROMUSCULAR

Levi and associates (61) noted a peculiar myalgic/asthenic syndrome in 17 of 18 patients receiving 5-azacytidine by intravenous bolus on a 200 mg/m² · day times five schedule. The onset was on the second to third day of therapy, with generalized muscle tenderness, weakness, and lethargy. As time progressed, the patients became confused and somnolent. They reported that one patient became comatose on two consecutive occasions after receiving the drug. The reason for this supposed toxicity

has not been established, but it has only been noticed sporadically by other investigators. The overall incidence of this neurologic-muscular syndrome in 745 patients given the drug was 2.7%.

OTHER TOXICITIES

Fever was a complication of 5-azacytidine therapy in 6% of patients receiving the drug. Elevation in temperature usually occurred 1 to 4 hours after administration of the drug, continued throughout the duration of the treatment course, and fell to normal limits within 24 h of completion of the course (55). A pruritic follicular skin rash has been noted in 2% of patients receiving 5-azacytidine (52). Stomatitis, phlebitis, and hypotension have been infrequently reported toxicities of 5-azacytidine.

Of the 745 patients receiving 5-azacytidine alone, there were six deaths that were thought to be drug-related (0.8%). One was due to hypotension, three to hepatic coma, and two secondary to thrombocytopenia.

Discussion

This paper represents the first review of laboratory, experimental animal, and clinical results on 5-azacytidine, a pyrimidine nucleoside analogue that has been in clinical trials since 1967. Although there are numerous studies on the mode of action of this drug, there has not been a final conclusion as to which mechanism plays the most significant role in the antineoplastic effect of the drug.

The antitumor activity of 5-azacytidine is high in experimental animal leukemia, while it is only marginal in experimental animal solid tumors. Interestingly, these results appear to correlate well with the initial clinical experience that indicates antitumor activity in human leukemia but generally disappointing activity in human solid tumors.

Comparative toxicology data show that qualitatively the beagle dog correctly predicted for bone marrow and hepatic toxicity in man but overpredicted for renal toxicity. None of the four animal species tested were predictive for the considerable nausea and vomiting that have consequently plagued clinical trials. Quantitively, there are tremendous interspecies differences, indicating that man can tolerate much higher doses of 5-azacytidine. Interspecies differences in the relative activity of kinases and deaminases probably account for this difference in tolerated dose. Further studies in this area would be desirable.

Pharmacokinetic data have shown that excretion patterns of radioactively labeled 5-azacytidine are the same in rodents, dog, and man. However, the paucity of published data does not allow us to compare metabolism of the drug in the various species.

5-Azacytidine has had a fairly extensive trial in several of the human solid tumors, but the therapeutic results have generally been disappointing. Further clinical trials in breast cancer, lung cancer, colorectal cancer, and melanoma do not seem warranted at this time. However, several other of the less common solid tumors have received inadequate trials, and more patients in these disease categories need to be treated with 5-azacytidine before the drug is labeled as inactive in solid tumors.

On the other hand, 5-azacytidine has a definite place in the treatment of acute myelogenous leukemia. The current treatment regimens for the primary treatment of acute myelogenous leukemia involve the use of various combinations of chemotherapeutic agents including cytosine arabinoside, 6-thioguanine, daunomycin, and occasionally adriamycin, and vincristine or prednisone, or both. Regimens using these agents in various combinations have given remission induction rates between 34% and 64%. One of the most important first-line combinations recently refined by the Cancer and Acute Leukemia Group B has been cytosine arabinoside plus daunomycin. Seventy-seven percent of patients with acute myelogenous leukemia have achieved

Table 5. Summary of the Major Toxicities of 5-Azacytidine

Dosage Schedule (Reference)	Range of Dosage	Total Patients Evaluated	Patients with Toxic Effects						
			Nausea	Vomiting	Diarrhea	Leukopenia <1500/mm³	Thrombocytopenia <100 000/mm³	Hepatic Toxicity: Abnormal LFT*	Hepatic Toxicity: Coma
		no.	%						
Weekly intravenously (53)	200-630 mg/m²	15	94	100	79	35	5	...†	...
Twice a week intravenously (48)	50-200 mg/m²	88	87	88	Occ‡	41	0	...	...
Daily intravenously × 5 (52-53, 61, 62, 67, 70)	50-400 mg/m²	376	71	70	60	24	9	2	0
Daily intravenously × 10 (56, 68)	0.5-2.4 mg/kg	206	76	93	60	27	29	21	0.7
Daily subcutaneously × 10 (69)	27-85 mg/m²	18	44	22	16	22	16	28	16
Continuous intravenous infusion daily × 5—fresh solution every 3 h (57)	50-200 mg/m² · day	36	58	58	Occ	76	25	...	...
Continuous infusion for 120 h—fresh solution every 4 h (58)	150-200 mg/m² · day	6	0	0	0	100	33	...	...

* LFT = liver function tests.
† No mention of this toxicity.
‡ Occ = occasional.

remission when treated with that combination (72).

Unfortunately, even with the high-induction rates of the above regimens, most patients do relapse, and the conventional antileukemic agents are soon exhausted. Our review has shown that 5-azacytidine has definite activity in these refractory cases of acute myelogenous leukemia of adults and children (36% response rate). The median duration of remission has been encouragingly long. In view of this documented activity, we would recommend that 5-azacytidine be used to treat acute myelogenous leukemia patients who have failed the more conventional agents, cytosine arabinoside, 6-thioguanine, and daunomycin. The role of 5-azacytidine earlier in the course of acute myelogenous leukemia has not yet been defined, although studies using 5-azacytidine in combinations as first-line treatment for acute myelogenous leukemia are ongoing.

Many areas worthy of future studies with this drug have been uncovered in this review. The protective effect on mice exposed to lethal X-irradiation needs to be confirmed. This could be important if the drug is used clinically with radiation therapy or total-body irradiation. The degradation products of the drug should be tested for experimental antitumor activity, as they may well be more active than the parent drug. Additional animal experiments using 5-azacytidine with cytidine as an antidote need to be done. This combination could theoretically be used to give a better therapeutic index (73).

Future clinical studies that are needed include the ongoing use of 5-azacytidine alone and in combinations for the first-line treatment of acute myelogenous leukemia. Further controlled explorations of the use of infusions of the drug versus other modes of administration would be of interest. Additional clinical trials using subcutaneous administration are important, as this could be a more desirable route, especially in children. The role of 5-azacytidine in consolidation, maintenance, and late intensification regimens in acute myelogenous leukemia remains to be defined.

Overall, 5-azacytidine is an effective drug for the treatment of patients with acute myelogenous leukemia who have relapsed after other therapeutic approaches. The full potential of this new drug is yet to be realized.

ACKNOWLEDGMENTS: The authors thank Drs. R. Alestalon, J. R. Bateman, J. Z. Finklestein, D. Hammond, J. A. Levi, K. D. McCredie, J. M. Quagliana, D. Schnider, and C. Tan for permission to use their previously unpublished data.

Received 18 December 1975; revision accepted 9 April 1976.

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